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Status of equine influenza virus in horses in accra metropolis in the Greater-Accra Region, Ghana

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Abstract

This study investigated the equine influenza status of horses within the Accra Metropolis. A sample of 100 horses randomly sampled from established horse stables in Shangrilla, Osu and Burma Camp were assessed. Nasal swabs were obtained from the nasopharynx of horses and subjected to reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). Data on stable management practices and horse characteristics were obtained to ascertain the risk factors associated with equine influenza virus. Data collected was subjected to descriptive statistics using Statistical Package for Social Sciences (SPSS) version 26.0. Out of the 100 horses sampled, equine influenza virus was not detected in any of the samples. This could be attributed to the type of housing facility for the horses and the non-exposure of the horses to the possible reservoirs such as donkeys in the study areas. Further studies should be conducted in different regions of Ghana in stable and free-range horses to ascertain the nationwide status of horses regarding equine influenza virus in Ghana.

Introduction

Horses are integral to transportation and income generation worldwide, significantly contributing to the socio-economic stability of their owners. They are employed to perform work, breeding, sports, and ceremonial activities-particularly in those countries where horses are culturally significant [1]. Despite their importance, the welfare of horses is often neglected, leaving them exposed to diseases such as the Equine influenza virus (EIV) [2]. Equine influenza virus (EIV) belongs to the family *Orthomyxoviridae;* in horses, it is considered one of the big causes of respiratory illness, with two antigenic subtypes currently identified: H7N7 and H3N8 [3]. The virus poses significant risks, including epidemics, reduced performance in sports, miscarriages, financial losses, and secondary infections [3]. In Ghana, the prestige of horse ownership has driven the importation of horses just as it occurs in Nigeria from various Western countries such as Argentina, Belgium, the Netherlands, Canada, USA, and United Kingdom [4]. This trend raises concerns about the possible introduction of the equine influenza virus into Ghana, especially since the virus is endemic in Europe and North America, with South America recognized as a significant source of the H3N8 strain [5]. Recently, outbreaks in Nigeria, South Africa, and Algeria showed that this virus was gaining ground in Africa, but little information on the virus in Ghana exists [5]. A report from OIE in 2020 recorded suspicion of EIV infection in Ghana due to outbreaks in some neighbouring countries including as Burkina Faso, Cameroon, Chad, Mali, and Ghana of which 66,000 horses and donkeys succumbed to the disease [5]. Equine influenza virus was detected using real-time polymerase chain reaction (RT-PCR) in 2018 and 2019 in Cameroon, Mali, Niger, Nigeria, Senegal, and Sudan following the outbreak; however, no case was confirmed in Ghana. Given the suspected presence of the virus in Ghana as a result of significant deaths due to equine influenza, it is crucial to verify the virus existence, even in the absence of clinical signs in horses. This study aimed to fill in the gap by investigating the presence of EIV in Ghana through molecular detection.

Methods

Study design: a quantitative research approach which involved a cross-sectional study design was used in this study. In this study, the purpose was to molecularly ascertain the infection status of horses in the Accra Metropolis concerning equine influenza virus and relate their infection status with management and animal (horse) factors.

Study area: the Accra Metropolis in the Greater Accra Region of Ghana served as the study area. The selected sites where the majority of the horses were kept included 3 main locations namely Shangrilla, Burma Camp, and Osu. These study sites were chosen because they have an

appreciable horse population that served as a representative population in obtaining the necessary sample size for this study.

Sampling and sample size: the horses to be sampled in the region of interest were selected using a simple random sampling method, which ensured that all horses had an equal chance of being selected and minimized sampling bias. The stables included horses of varying ages, breeds, and genders. Using Fosgate's sample size table [6], the required sample size was 76 horses from a total population of 297 horses (200 at Shangrilla, 33 at Osu, and 67 at Burma Camp). In order to improve the reliability of the study, 100 horses were ultimately sampled.

Nasal sample collection: prior to nasal swabs collection, the horses were held under proper restraint to avoid injury to the horses and the veterinarians. Sterile nasal swabs were rolled through the nasal cavity along the nasal septum through to the nasopharynx. Nasal swabs samples collected from each horse was placed into tubes containing one millilitre (1ml) of viral transport medium (VTM). All the nasal swab samples collected were transported in cold chain (use of ice packs were used in the cooler to maintain the temperature) to the Accra Veterinary Laboratory for RNA extraction and Reverse-Transcription Quantitative Polymerase Chain Reaction (RT-qPCR).

Animal parameters and horse stable management practices: animal parameters including age, sex, body condition score, breed, and location of the horses were collected during the nasal samples collection. Data on horse stable management practices including the type of housing for the horses, the type of feeding management, vaccination against equine influenza, vitamin supplementation, cleaning and disinfection of horse stables, and the quarantine and monitoring of new stock of horses were also obtained.

Laboratory analysis

RNA extraction procedure and reverse-transcription quantitative polymerase chain reaction (RT-qPCR): the RNA extraction was conducted using the procedure previously reported by Alsulaimany *et al*. [7]. The nasal swab samples were prepared by vortexing and 200µl of each sample was then treated with 200µl Proteinase K and 255µl KSB buffer before incubation at 56°C for 10 minutes. This was then centrifuged at 10,000 revolutions per minute (rpm) for 1 minute. After centrifugation, 350µl of ethanol was added, and the mixture was transferred to a spin column for a series of centrifugations and washed with 500µl of KSW1 and 500µl of KSW2 buffers. In addition, 70 µl of KSE buffer was added directly at the centre of the spin columns and incubated for 1 min at room temperature and then centrifuged for 1 min at 10000rpm. The final RNA extracts were stored at -20°C until further analysis. Total nucleic acid was extracted using a QuantiTect Multiplex RT-PCR Kit and tested for Influenza A RNA via RT-qPCR, following the protocol by Alsulaimany *et al*. [7]. The PCR process involved specific primers and a probe, with thermal cycling conditions set for denaturation, annealing, and fluorescence reading. The sequence of the primers used included Primer M25-F: 5´-AGATGAGATCTTCTAACCGAGGTCG-3´ (MWG-Operon), Primer M124-R: 5´-TGCAAAAACATCTTCAAGTCTCTG-3´ (MWG-Operon). The probe sequence used for this study was Probe FAM_MG5 5´-TCAGGCCCCCTCAAAGCCGA-3´. Thermal cycling was performed at a temperature of 50^{o}C for 20 minutes. Initial denaturation was performed at a temperature of 95^{o}C for 15 minutes. Denaturation and annealing were performed at 94°C and 60°C respectively for 45 seconds. This was followed by fluorescence reading after 1 hour.

Data analysis: descriptive statistics was carried out on data obtained using Statistical Package for Social Sciences (SPSS) Version 26.0 software.

Results were presented in tables using percentages and frequencies.

Results

Prevalence of equine influenza in horses: the findings on the prevalence of equine influenza virus in horses showed that out of the 100 horses sampled and tested, none of the horses tested positive for equine influenza virus; indicating a zero (0%) prevalence (Table 1).

Horse stable management practices: the findings displayed in Table 1 revealed that all of the stables (100%) used the closed stable to house their horses. In addition, the stable managers did not vaccinate their horses against the equine influenza virus. Veterinary supervision, regular vitamin supplementation, quarantine and monitoring of new horses, and zero grazing were practiced at all the horse stables.

Discussion

This current study being the first report on equine influenza in Ghana focused on assessing the equine influenza status of horses in the Accra Metropolis in Ghana, and did not detect the presence of equine influenza virus. This finding is consistent with Alsulaimany *et al*. [7], who also reported no detection of equine influenza virus using RT-qPCR in horses from Saudi Arabia. In contrast, Diallo *et al*. [5] identified EIV in horses from West African countries, including Nigeria, Gambia, Mali, and Chad, using the same method. Similarly, Boukharta *et al*. [8] detected the H3N8 strain in Moroccan horses, which differs from our results in Accra. The discrepancy may be due to the timing of sampling, as Diallo *et al*. [5] collected samples during a 2019 outbreak, increasing the likelihood of virus detection. The absence of EIV in this study might also be attributed to the good management and biosecurity practices at the stables, similar to the findings by Alsulaimany *et al*. [7], who found no positive cases in well-managed horse farms. Research has shown that rigorous biosecurity practices can significantly reduce the spread of equine influenza [5], which could have influenced the outcomes of our study.

In addition, zero-grazing and individually housing horses in closed stables, where each horse has its own stall, are effective management practices that likely played a significant role in preventing the transmission of equine influenza virus (EIV) in this study, as highlighted by Alsulaimany *et al*. [7]. High population density, whether in stables or during field grazing, increases the risk of EIV transmission, as virus-laden aerosols from infected horses can travel over 35 meters [3]. The implementation of a quarantine for new horses in this study likely further reduced the risk of EIV infection, underscoring the importance of proper monitoring to prevent outbreaks [8]. Additionally, regular vitamin supplementation, particularly with Vitamin D, has been shown to enhance immune function and lower the risk of respiratory infections, including EIV, by maintaining cellular integrity, inactivating viruses, and reducing the production of proinflammatory cytokines [9].

The policy of not vaccinating horses against equine influenza virus in Ghana is an effective measure to prevent the introduction of the virus to horses in the Accra Metropolis. This is supported by evidence from Watson *et al*. [10], who noted that failed vaccinations played a role in the 2007 equine influenza outbreak in Australia. Authorities in Australia believed that the equine influenza virus escaped a quarantine station due to lax biosecurity protocols. In addition, many horses certified as vaccinated against EIV had no protective antibodies, suggesting poor immune response, lack of vaccination compliance, and or ineffective or failed vaccines [10]. Despite the outcome of this study, the study is limited by the absence of serological testing which could have given the immunity status of these horses whether they have been vaccinated or not against equine influenza virus. In addition, the source of the horses sampled did not include horses from local individuals who might not be in well-managed and

structured housing conditions with relatively good biosecurity services.

Conclusion

This study represents the first investigation into the presence of equine influenza virus in horses within the Accra Metropolis, Greater-Accra Region of Ghana, with no detection of the virus. The absence of equine influenza virus may be attributed, in part, to the rigorous stable management practices implemented by the horse stable managers at the study locations. It is recommended that further studies should be conducted in different regions of Ghana in stable and free-range horses in order to ascertain the nationwide status of equine influenza virus in Ghana. In addition, serology to detect the immune status or vaccination status should be carried out in different seasons of the year to ascertain the effect of the season in the EIV prevalence in the study area.

What is known about this topic

- *Equine influenza virus is a significant cause of respiratory illness in horses, with two main antigenic subtypes (H3N8 and H7N7) recognized, leading to substantial economic losses in the equine industry due to its highly contagious nature and potential for outbreaks;*
- *There is the presence of EIV in neighbouring West African countries; which indicates the potential for transboundary transmission and highlighting the importance of monitoring equine health in the region;*
- *Effective management practices, including rigorous biosecurity measures and vaccination protocols, have been shown to significantly reduce the incidence and spread of equine influenza virus infection.*

What this study adds

- *This study adds valuable insight regarding the possible absence of equine influenza virus in horses in the Greater Accra Region of Ghana despite positive confirmations in neighbouring West African countries;*
- *The absence of equine influenza virus detection in this study suggests that good management practices and biosecurity measures implemented in the horse stables may have played a significant role in preventing the transmission of the virus;*
- *The study further highlights the importance of regular surveillance and emphasizes the need for comprehensive studies across different regions of the country to ascertain the nationwide prevalence of the virus in both stable and free-range horses.*

Competing interests

The authors declare no competing interests.

Authors' contributions

The authors of this study contributed to the research in the following manner: Derrick Adu Asare, Kadima Bakau Kwem, and Benjamin Obukowho Emikpe were responsible for conceptualizing the study and designing the methodology. Derrick Adu Asare, Stephanie Osei Bediako, David Rodgers, Hope Richard Otsyina, were responsible for data collection, analyzing the data, and drafting the original manuscript. Theophilus Odoom, was responsible for laboratory analysis. Derrick Adu Asare, Kadima Bakau Kwem, Benjamin Obukowho Emikpe, and Edmond Onidje, contributed to final data analysis, as well as reviewing and editing the manuscript. Benjamin Obukowho Emikpe, Derrick Adu Asare, and Edmond Onidje reviewed and edited the final manuscript.

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Table

Table 1: prevalence of equine influenza virus and stable management practices

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Table 1: prevalence of equine influenza virus and stable management practices			
Location of horse	**Number of horses sampled**	**Equine influenza status**	
		Positives (N, %)	Negatives (N, %)
Shangrilla	50	0 (0%)	50 (100%)
Burma camp	29	0 (0%)	29 (100%)
Osu	21	0 (0%)	21 (100%)
Overall prevalence	100	0 (0%)	100 (100%)
Horse stable management practices			
Variable	**Categories**	**Frequency**	**Percentage (%)**
Type of horse stable	Closed stable	3	100%
Equine influenza vaccination	No	3	100%
Regular vitamin supplementation	Yes	3	100%
Quarantine and monitor new horses	Yes	3	100%
Presence of veterinary supervision	Yes	3	100%
Type of feeding	Open-field grazing	0	0%
	Zero grazing	3	100%